

IPEA/US

PCT/US04/18783.25022005

1. A synthetic, non-cytopathic negative-strand RNA virus replicon comprising
  - a) a nucleotide sequence of said RNA virus, wherein the sequence of one or more structural genes is inactivated or deleted; and
  - b) a nucleotide sequence encoding a selectable marker suitable for selection, wherein said sequence encoding a selectable marker is under the control of the RNA virus replication machinery and wherein the replicon, when introduced into a host cell, allows production of a stable culture of cells containing the replicon.
2. The replicon of claim 1, wherein said sequence encoding a selectable marker is a gene that confers resistance to an antibiotic.
3. The replicon of claim 2 wherein said gene is a *bsd* gene.
4. The replicon of claim 1, further comprising a sequence encoding a heterologous protein.
5. The replicon of claim 1 further comprising a reporter gene.
6. The replicon of claim 5, wherein said reporter gene is a gene encoding green fluorescent protein (GFP).
7. The replicon of claim 1 wherein said RNA virus is respiratory syncytial virus (RSV).
8. The replicon of claim 7, wherein the sequence encoding the F, G and SH glycoproteins is deleted.
9. The replicon of claim 8 wherein said sequence encoding a selectable marker is a gene that confers resistance to an antibiotic.
10. The replicon of claim 9, wherein said gene is a *bsd* gene.
11. The replicon of claim 10, further comprising a reporter gene.

IDEA/US

12. The replicon of claim 11 wherein said reporter gene is a gene encoding GFP.
13. A cell line comprising the replicon of claim 1.
14. The replicon of claim 12, wherein said replicon is harbored in a cell line selected from the group consisting of BHK-RR-B51E (ATCC deposit number PTA-5257) and HeLa-RR-B51S (ATCC deposit number PTA-5258).
15. The replicon of claim 12, further comprising a sequence encoding a heterologous protein.
16. A cDNA of a non-cytopathic negative-strand RNA virus replicon comprising
  - a) a nucleotide sequence complementary to the genome of said RNA virus, wherein the sequence encoding one or more structural genes is inactivated or deleted;
  - b) a nucleotide sequence comprising a heterologous promoter sequence operatively linked to said sequence of a); and
  - c) a nucleotide sequence comprising a gene encoding a selectable marker suitable for selection, wherein said gene is under the control of the RNA virus replication machinery and wherein the cDNA, when introduced into a host cell, allows production of a stable culture of cells containing the replicon.
17. The cDNA of claim 16, wherein said heterologous promoter sequence is selected from the group consisting of T7 polymerase promoter, cytomegalovirus immediate early promoter, SV40 early promoter and polymerase I promoter.
18. The cDNA of claim 16 wherein said promoter is a T7 polymerase promoter.
19. A replicon encoded by the cDNA of claim 16.
20. A method comprising
  - a) transfecting a cell line in culture with a polynucleotide comprising

TPA/US

i) a DNA sequence complementary to a negative-strand RNA virus replicon, wherein the sequence encoding one or more structural proteins is inactivated or deleted;

ii) a DNA sequence comprising a gene encoding a selectable marker protein suitable for selection;

b) culturing said cell line *in vitro*;

c) selecting for cell populations displaying the phenotype conferred by said selectable marker, thereby producing a stable culture of cells containing the negative-strand RNA virus replicon; and

d) isolating RNA virus sequences from said cell populations of c).

21. The method of claim 20, wherein said selectable marker is a gene that confers resistance to an antibiotic.

22. The method of claim 21, wherein said antibiotic is blasticidin.

23. The method of claim 21, wherein said selecting of c) comprises culturing said cell line in a medium containing an antibiotic.

24. The method of claim 20, wherein said RNA virus is RSV.

25. The method of claim 24, wherein said RSV sequence comprises a mutation or deletion that renders the F, G and SH glycoproteins inoperative.

26. A method comprising

a) transfecting a cell line in culture with

i) a DNA sequence complementary to a negative-strand RNA virus replicon, wherein the sequence encoding one or more glycoproteins is inactivated or deleted and wherein said sequence comprises a T7 polymerase promoter operatively linked to said sequence of I), and wherein said sequence further encodes a selectable marker; and

ii) a DNA sequence encoding a T7 polymerase;

b) culturing said cell line *in vitro*;

IPED/W

c) selecting for cell populations displaying the phenotype conferred by said selectable marker thereby producing a stable culture of cells containing the negative-strand RNA virus replicon; and

d) isolating RNA virus sequences from said populations of c).

27. The method of claim 26, wherein step a) further comprises transfecting said cell line with support plasmids encoding viral proteins necessary for replication and mRNA synthesis.

28. A method for mobilizing a negative-strand RNA virus replicon comprising

- a) transfecting the cell line of claim 13 with a plasmid encoding a viral glycoprotein that allows virion formation;
- b) culturing said cell line of a) in culture medium;
- c) inoculating a fresh cell line with virions present in the culture medium of b).

29. The method of claim 28 wherein said viral glycoprotein that allows virion formation is a VSV G protein.

30. The method of claim 28, wherein said selectable marker is a gene that confers resistance to an antibiotic, said method further comprising

- d) culturing said inoculated cells of c) on medium containing the antibiotic; and
- e) identifying replicon-expressing cells from the surviving cells.

31. A method comprising culturing a cell line containing the replicon of claim 4 in vitro to produce said heterologous protein.

32. A method for screening for antiviral agents comprising

- a) contacting the cell line of claim 13 with a candidate agent, and
- b) testing for an increase or decrease in replication or activity of the RNA virus replicon relative to a control cell line harboring the same replicon, but which control cell line has not been contacted with the candidate agent.